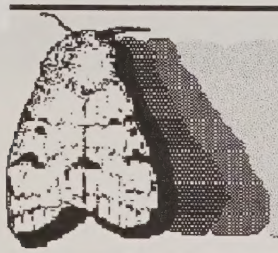
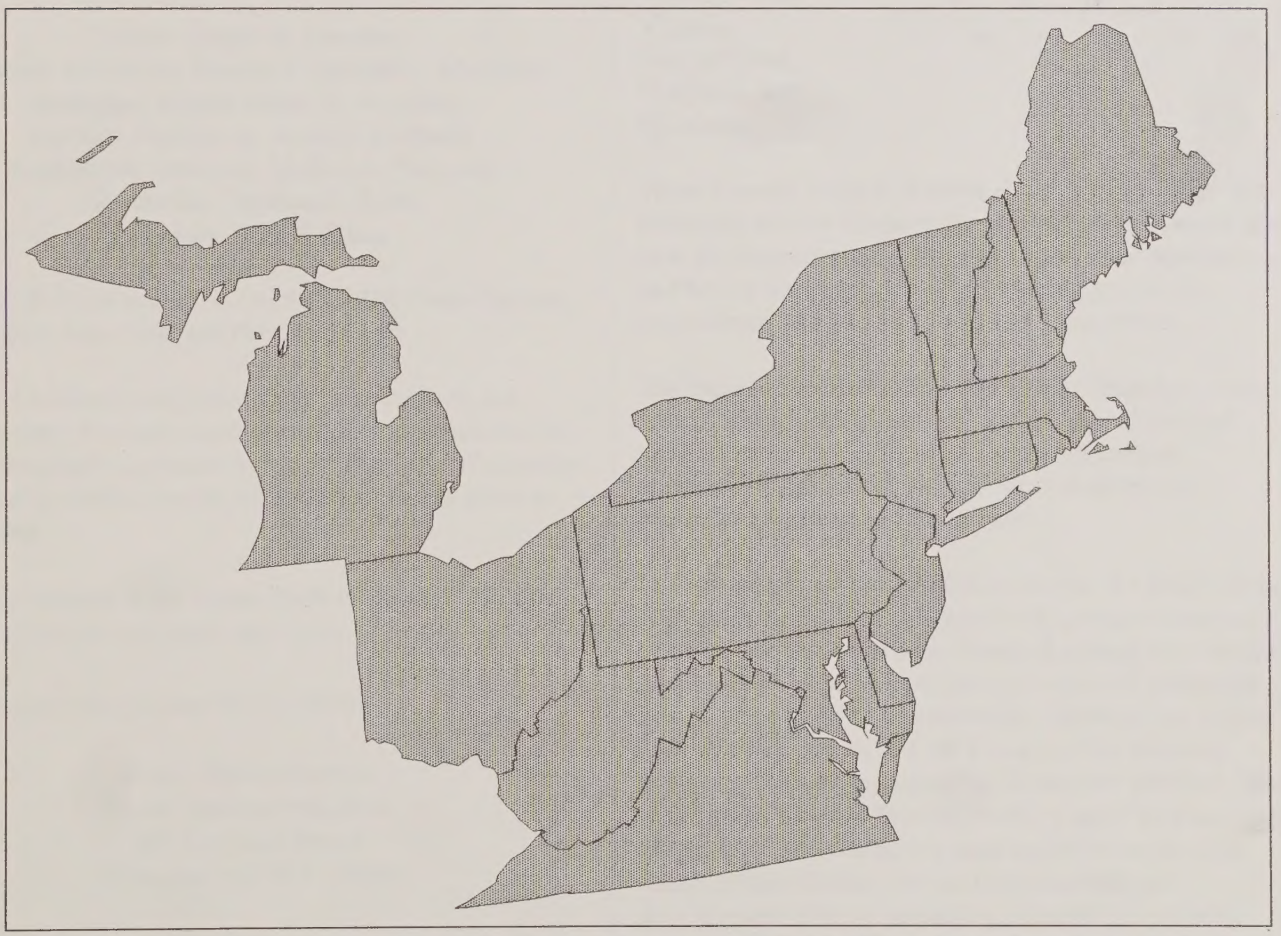


Gypsy Moth News

October 1995 Issue Number 39

My oh maimaiga!



Entomophaga maimaiga has been found in these States since 1989. Significant larval mortality caused by E. maimaiga occurred during 1995 throughout New Jersey, Pennsylvania, Maryland, West Virginia, Virginia, and Michigan.

FOREST SERVICE U.S. DEPARTMENT OF AGRICULTURE

NA NORTHEASTERN AREA State and Private Forestry

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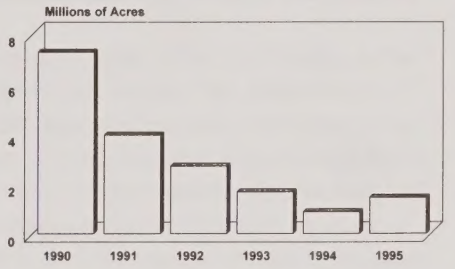
From the Editor

This issue of the *News* contains a lot of information about the fungus *E. maimaiga* and the nucleopolyhedrosis virus (NPV) both of which are responsible for significant declines in gypsy moth populations throughout the East. Evidence of the effect of these population regulating factors has been dramatic in parts of Virginia, West Virginia, Pennsylvania, Maryland, and Michigan.

Gypsy Moth Defoliation, 1990-1995



There is much yet to be learned about *E. maimaiga*. The following articles illustrate the need for more research about how the fungus spreads, its effect upon other lepidoptera, and how it can be used to help prevent gypsy moth populations from reaching outbreak proportions.

The literature review of NPV by Onken illustrates a long road of virus isolation, testing, and attempted product development. Yet, still today, we do not have a commercially available virus product to offer as an alternative to chemical insecticides.

Unfortunately, as research dollars shrink, the ability of the USDA Forest Service to aggressively pursue continued research into these avenues of natural control also shrinks. But that shouldn't diminish the importance of continued investigations into these potentially useful natural control agents. *E. maimaiga* and NPV may well be the most promising population regulating factors we will find. More to the point, they are literally "birds in hand" that we should not ignore when it comes to looking for alternatives to chemical insecticides. To continue research and development of these promising population regulating agents may require a greater sense of urgency and focus. If, after reading the following articles about *E. maimaiga* and NPV, you think more effort is needed, you should contact: Robert Lewis, Station Director, Northeastern Forest Experiment Station, 5 Radnor Corporate Center, Suite 200, Radnor, PA 19087-4585.

--DBT

Entomophaga maimaiga in North America: A Review

Richard Reardon, USDA Forest Service, Northeastern Area State and Private Forestry, Morgantown, WV, and Ann E. Hajek, Cornell University (formerly with the Boyce Thompson Institute), Ithaca, NY

Article adapted from an IPM Technology Transfer publication entitled, "*Entomophaga maimaiga* in North America: A Review", USDA Forest Service, NA-TP-15-93, dated September 1993.

Introduction

In eastern North America, the gypsy moth is subject to a variety of naturally occurring infectious diseases caused by several kinds of bacteria, fungi, and a nucleopolyhedrosis virus (NPV), which was inadvertently introduced with gypsy moth or its parasites. There are six endemic species of entomopathogenic (causing disease in insects) fungi known to infect the gypsy moth.

The fungal class Zygomycetes, which includes the bread molds, is a primitive group of fungi with no species native to North America known to infect gypsy moth. Species in one zygomycete order, the Entomophthorales, are predominantly insect pathogens. Many entomophthoralean pathogens are known to cause dramatic epizootics (disease outbreaks) in insect populations.

In Japan, epizootics of an entomophthoralean fungus have frequently been reported from high density populations of gypsy moth. In 1908, pest managers attempting to control expanding gypsy moth populations in the Northeast first heard of this fungus. Gypsy moth larvae infected with this entomophthoralean fungus were collected near Tokyo, Japan, in 1909 and brought to the United States for evaluation as a gypsy moth control. This fungus appeared to be a member of the *E. aulicae* species complex. In 1910-1911, larvae infected with the "gypsy fungus" were released at six sites near Boston. No transmission of this disease resulted due, in part, to unfavorable weather conditions and the occurrence of a virus outbreak. When the local gypsy moth population collapsed in 1911, there was no way to continue propagating the fungus (the gypsy moth could not be reared in the laboratory over the winter and the maintenance of fungal cultures failed due to reliance on overwintering resting spores produced by late instar larvae). Therefore, this project was discontinued.

In 1984, researchers isolated this entomophthoralean fungus from the Asian gypsy moth in Japan and brought isolates to the United States. Stages of this fungus now could be maintained year round in the laboratory using several different culture media, rather than having to be perpetuated on gypsy moth larvae. The morphological characteristics of the Japanese isolates were identical to *E. aulicae* strains, yet only the Japanese fungus could infect gypsy moth. Since the isozyme pattern, distribution, and host range of this fungus differed from those of other isolates within the *E. Aulicae* species complex, the name *Entomophaga maimaiga* was given to the Japanese isolates. The specific name for this new species, "maimaiga," was based on the Japanese common name for the gypsy moth.

Disease Symptomatology

E. maimaiga and NPV are the principal natural enemies of gypsy moth that kill large numbers of larvae. Cadavers of larvae killed by both diseases remain hanging on tree boles. Cadavers of late instar larvae killed by *E. maimaiga* are often oriented vertically with heads down, all prolegs frequently at a 90-degree angle to the axis of the body, and bodies tightly attached to tree boles. Larvae recently killed by the fungus are soft-bodied, and older cadavers appear dry. By contrast, larvae killed by NPV are predominantly positioned with anterior prolegs attached to the bole, the anterior section of the body hanging unattached, and the body bending at an angle of less than 90 degrees. These external characteristics described above are not sufficiently reliable for diagnosis; therefore, larvae should be dissected.

Distribution (1989-1995)

Epizootics of *E. maimaiga* have occurred in New England and parts of mid-Atlantic gypsy moth populations. The distribution of this fungus continues to expand in areas more recently colonized by gypsy moth. Introduction efforts along the southern and western edges of gypsy moth distribution have obscured the rate of natural spread of *E. maimaiga*, although spread of greater than 1-km was recorded between consecutive years at numerous release sites. This fungus is now so widespread in Virginia and Michigan that it is difficult to determine whether the fungus at an individual location is the result of spread from a

release or of natural migration from the north where it is established.

When *E. maimaiga* was first found in North America in 1989, the simplest explanation was that the 1910 and 1911 introductions had been successful in establishing the fungus, which later produced local infections resulting in its subsequent spread. *E. maimaiga* may not have been detected between 1911 and 1989 because cadavers of larvae killed by *E. maimaiga* look similar to cadavers of larvae killed by NPV to untrained observers. The rapid spread of *E. maimaiga* documented from 1989 to 1992 poses questions regarding the successful establishment of this fungus during releases in 1910 and 1911. If the fungus can spread as quickly as recorded from 1989-1992, it is difficult to imagine that it required 79 years to spread from Boston to its 1989 distribution. The strain of *E. maimaiga* introduced in 1910-1911 was considered weak, and the introduction project was considered a failure and discontinued. The strain of *E. maimaiga* introduced in 1910-1911 may have barely survived in the environment for many years, and then a more highly pathogenic strain evolved and began spreading. Another hypothesis would be that *E. maimaiga* did not establish at all in 1910-1911, but was more recently accidentally introduced to North America.

Use as a Mycoinsecticide

Spread.--The relocation of *E. maimaiga* resting spores and its habitat soil from one location to another requires obtaining the necessary permits from the USDA Animal and Plant Health Inspection Service (APHIS) and taking additional precautions to ensure that plant pathogens (e.g., *Armillaria mellea* rhizomorphs) and arthropod pests are not unintentionally spread.

Therefore, laboratory production of *E. maimaiga* on artificial media would provide a method of producing quantities of *E. maimaiga* without the potential of introducing pest species in soil.

Constraints on operational use.--Numerous constraints limit the development of entomopathogenic fungi for use as mycoinsecticides. Foliar applications of fungi are very sensitive to abiotic factors (humidity, degradation by ultraviolet light and solar heat, removal from target habitat by rainfall). Compared with insecticides, fungi can be relatively slow to kill the host (at least 1 week). Fungi are also often short-lived in storage and relatively expensive to produce. Host specificity of many entomophthoralean fungi make their production for control more expensive although making them more desirable due to decreased nontarget impact. Dried-mycelium preparations will present unique formulation problems of adhering the larger particles to leaf surfaces while protecting them from adverse environmental conditions.

Research Needs

Additional research is critically needed in the areas of field ecology, biology, and population dynamics of *E. maimaiga*, before mycoinsecticide development and field application. There are many unanswered questions concerning *E. maimaiga*. What factors trigger germination of resting spores in various micro habitats, influence larval infection and disease incubation period, and affect spatial and temporal patterns of spore dispersal? How are disease transmission and spread influenced by host and pathogen densities and biological interactions between this fungus and NPV? The effects of *E. maimaiga* on nontarget organisms are being evaluated further; these data are a first-order requirement for development of any control agent.

E. maimaiga is effective in both high- and low-density gypsy moth populations, unlike the nucleopolyhedrosis virus, which is only effective at high-density populations. The fungus could play a significant role in the natural control of gypsy moth, especially in years with a wet spring. Only time will tell whether increasing the area where *E. maimaiga* is established will lead to constant lower populations of the gypsy moth in North America.

Copies of the entire publication can be obtained by contacting Richard Reardon at 304-285-1566.

Effects of *Entomophaga maimaiga* on Non-Target Insects

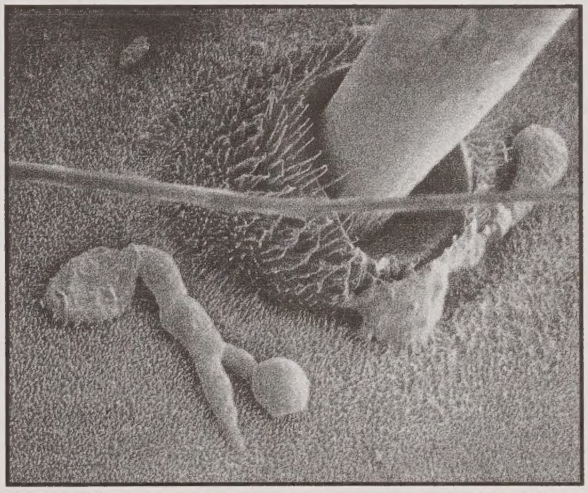
Ann E. Hajek, Department of Entomology, Cornell University, Ithaca, NY

Studies published in 1988 demonstrated that a strain of *E. maimaiga* collected in Japan in 1984 only infected Lepidoptera. These studies tested relatively few species of Lepidoptera, predominantly from laboratory colonies. Among the lepidopteran species tested other than gypsy moth, high levels of infection were found only in lymantriids (including Douglas fir tussock moth); and low levels of infection were found in a few of the noctuids and arctiids.

Beginning in 1992, Linda Butler (West Virginia University), Dick Reardon (FS, Morgantown, WV), and I cooperated on studies evaluating whether northeastern U.S. strains of *E. maimaiga* would infect species of Lepidoptera native to Appalachian forests. Laboratory bioassays were conducted over two years to maximize the diversity of species that were tested. These laboratory tests optimized chances for *E. maimaiga* to cause infections, yielding information about an idealized host range for this pathogen. Lepidopteran larvae were field-collected or reared from eggs obtained from females. Larvae were reared on foliage and then challenged by externally inoculating them with *E. maimaiga* conidia. Infection was recorded when species being tested died and produced fungal spores.

Out of a total of 78 species tested from 10 different superfamilies, cadavers of 35.6 percent of the species produced *E. maimaiga* spores. Infections occurred in 7 of the 10 superfamilies tested although infection levels were <50 percent for all superfamilies except Bombycoidea, Sphingoidea, and Noctuoidea. Only one species became infected at >50 percent within both the Bombycoidea (*Malacosoma disstria*) and Sphingoidea (*Manduca sexta*). In the Noctuoidea, >50 percent infection was only found within the Lymantriidae. We found that among the many species that were not infected by *E. maimaiga*, lack of infection was frequently due to inability of the fungus to penetrate the larval cuticle, although fungal cells were able to develop within the hemocoel if the cuticle was bypassed. We know that surface structure can be used by pathogenic fungi to detect hosts. In agreement, an analysis of all species tested demonstrated lower levels of infection among caterpillars with little surface sculpturing and less setation.

Data from laboratory bioassays are frequently used to evaluate host range for insect natural enemies. However, laboratory bioassays optimize chances for infection and frequently do not agree with field observations. Therefore, during 1995, we continued our studies by investigating the host range of *E. maimaiga* under field conditions. With the help of Steve Talley (Rockbridge County, Virginia), we sampled larvae of gypsy moth and non-target Lepidoptera in seven plots in Virginia where we knew *E. maimaiga* was present and active. The moderate density gypsy moth populations in these plots experienced 40.8 - 97.5 percent infection by *E. maimaiga* during the field season. A total of 1421 larvae from 53 species belonging to 7 lepidopteran families and 4 subfamilies were collected and reared. Only two individuals, one of 296 *Malacosoma disstria* (Bombycoidea: Lasiocampidae) and one of 96 *Catocala ilia* (Noctuodea: Noctuidae) became infected by E. maimaiga. Unfortunately *C. ilia* had not been tested during laboratory bioassays. In summary, laboratory studies demonstrated infection by *E. maimaiga* over a greater diversity of species compared with field studies, and for those species infected in both the laboratory and field, the percent of infection was much higher in the lab studies than findings from the field.



Two conidia that have germinated and are penetrating through gypsy moth cuticle at the base of a seta. The rounded structures are the penetrating structures (appressoria), while the collapsed, oblong structures are the conidia that have germinated. The connections in between are called germ tubes.

Gypsy Moth Fungus, *Entomophaga maimaiga*, Projects Funded by the USDA Forest Service

Principal Investigator and Status	Title and Objective(s)
Ann Hajek Cornell University (formerly of Boyce Thompson Institute) Started 4/15/92 Status - ongoing	**Impact of the fungus *E. maimaiga* on nontarget Lepidoptera - Lab/Field Studies** 1. To evaluate the host range of northeastern isolates of *Entomophaga maimaiga*, with emphasis on the endemic lepidopteran fauna of West Virginia and Virginia. 2. To determine the seasonality of resting spore activity in the field
Joe Elkinton University of Massachusetts Started 9/9/91 Status - Terminate 4/4/96	**Competition of gypsy moth nucleopolyhedrosis virus and the fungus *E. maimaiga*** 1. Assess the relative competitive abilities of gypsy moth NPV and *E. maimaiga* using small scale "bugs-in-bags" experiments. 2. Directly test whether *E. maimaiga* can out compete NPV in the field.
Ann Hajek Cornell University Started 8/15/90 Status - Ongoing	**Spread of *E. maimaiga* in the Southern Appalachians** 1. Introduce *E. maimaiga* into gypsy moth populations at a few locations in the Appalachian area and evaluate its impact. 2. Determine the rate of spread of *E. maimaiga* from areas of introduction, and those abiotic and biotic factors influencing spread. 3. Compare several methods to optimize establishment of *E. maimaiga* in areas where it does not now occur.
Barry Hunter California University of PA Started 5/13/93 Status - Terminated 9/30/94	**Separation and purification of *E. maimaiga* resting spores from gypsy moth cadavers** Isolate resting spores of *E. maimaiga* from other microscopic forms and their metabolic products using field collected gypsy moth cadavers.

Gypsy Moth Nucleopolyhedrosis Virus (NPV) Literature Review

Amy Onken, USDA Forest Service, Northeastern Area State and Private Forestry, Morgantown, WV 26505

The gypsy moth nucleopolyhedrosis virus (NPV) is a member of the genus Baculovirus and is totally unrelated to viruses that cause disease in humans and other animals. The origin of gypsy moth NPV in North America is unclear, but it is speculated that since all life forms of gypsy moth may contain a low level of NPV, it was present in the samples brought to Medford, Massachusetts, from Europe by Leopold Trouvelot in 1864. Recognized in the early 1900's, the gypsy moth disease caused by NPV was often referred to as "wilt" due to the appearance of diseased larvae. As early as 1911, Reiff wrote "I am quite convinced that we can apply the wilt in a systematic manner to the benefit of our forests, and that in so doing we shall come considerably nearer to a solution of the problem of destroying the gypsy moth". Here, in the last quarter of the 20th century, efforts are still being made to cope with the gypsy moth problem and to make use of the gypsy moth NPV.

Biology

Gypsy moth NPV is a natural component of the host's habitat where it persists in soil, litter, and bark. The disease can reach epizootic proportions as gypsy moth population densities increase. These epizootics result from increased transmission rates, within and between generations, as small larvae become infected and die on leaves. Healthy larvae become infected by eating contaminated foliage and become factories that produce rod-shaped virus particles. In the infection process, the metabolism of the cells in the infected insect is directed by the virus to produce more virus. The material used to form additional virus comes from the cell itself, and the process results in death of the cell. The virus rods formed become surrounded by or occluded within a many-sided structure, the polyhedral occlusion body (OB). The major function of the OB is to protect the viral rods it encloses from physical, chemical, and biological agents. In the gut of the insect, the OBs dissolve and release the viral rods, which first cross the gut wall and then infect the hemocytes (blood cells). The disease progresses to the internal tissues and organs of the larva, eventually causing a general viral infection. An infected larva will demonstrate characteristic symptoms of disease which include loss of appetite, listlessness, a darkening in color, a moist appearing integument, and often a tendency to climb upward. Infected larvae usually die within 9-11 days and characteristically hang by their prolegs from foliage or bark in an inverted "V" position. An infected cadaver essentially becomes a fragile sac of OB's and when ruptured, its contents are spilled onto foliage and bark, thus providing a source of inoculum for infecting healthy larvae.

Gypsy moth NPV can also be transmitted from generation to generation via the eggs. The current theory that is generally accepted by most researchers involves the following sequence of events: the NPV infected female moth contaminates her eggs, either externally or internally, with NPV. Larvae hatching in the succeeding generation are either already infected or become so after consuming OBs on the surface of the egg shell. These infected larvae die in the first instar and become the source of NPV inoculum for the healthy larvae in the population. Healthy larvae of all stages then contract the disease by feeding on NPV-contaminated foliage. Some female larvae that ingest OB's and that are close to pupation do not die of the disease, but rather, through some mechanism as yet not clearly defined, transmit NPV to their eggs. In many dense gypsy moth populations, the virus kills in excess of 90 percent of the larvae and reduces populations to levels where they cause only minimal defoliation and tree damage in the same year.

Development of the Gypsy Moth NPV Product, Gypchek

In the late 1950's, the USDA Forest Service (FS) began to explore the feasibility of developing the gypsy moth NPV. From early field tests and from a variety of laboratory studies conducted on gypsy moth NPV and other insect viruses, it became clear that, gypsy moth NPV was not one of the most virulent insect viruses and it remained active for only a few days following foliar application. In the late 60's to early 70's, research revealed that NPV's had the following unique characteristics that must be taken into account when using them: (1) NPV must be eaten to be effective and the feeding activity of the target pest must not be impaired; (2) NPV is quickly degraded by ultraviolet radiation, so the formulations must provide ultraviolet protection; (3) the feeding area (leaf surfaces) must be thoroughly covered to maximize ingestion of the virus; (4) leaf expansion should be well advanced to ensure minimal untreated expansion areas; (5) the coverage of the target area must remain in place for several days to ensure contact between the NPV and the host insect; and (6) the susceptibility of the gypsy moth larva for a given dose (i.e. dose in relation to weight)

decreases rapidly as it grows larger. With these unique characteristics in mind, research from the late 60's to early 70's focused on finding the most virulent gypsy moth NPV, developing a cost effective NPV production system, and finally developing a tank mix with sunlight protective properties.

To find the most virulent gypsy moth NPV required the evaluation of NPV material from around the world as well as isolates obtained from various sections of the United States (U.S.). NPV strain selection was quite difficult because several reports indicated wide variation in the potency of NPV isolates tested against larvae of the same instar. Other complications were that different geographic populations of the insect responded differently to the same virus source. Similar variations were reported for several virus sources tested against a single gypsy moth population. The selection for the most virulent strain of NPV was done to assure that the strain developed was the most effective for insect populations in the Northeastern U.S., and that the strain developed met identity criteria of EPA and was not a material of mixed heritage and potency. The strain selected for development, the Hamden strain, was a compromise and generally exhibited stronger potency against U.S. populations. This strain was isolated from a Connecticut population affected by a natural epizootic of NPV and has been used in many field and laboratory studies.

By 1972 research had progressed to the point where field testing of the new and improved NPV product was appropriate.

On May 24, 1974, the first aerial application of virus was made at the rate 1 x 10^{12} OB's per 0.4 ha. The spray formulation consisted of the following materials: a commercial adjuvant-extending material Shade® (International Mineral Corporation, Chicago, IL), a sticker spreader Chevron®, a stabilized molasses material, CIB® (Cargill Insecticide Base), NPV, and water. Each 0.4 ha received 7.2 liters of finished spray material. Application of the virus provided foliage protection, population reduction, and increased the number of virus-infected larvae.

In 1975, the FS contracted with Bio-Serv, Inc. for the production of gypsy moth virus. While Bio-Serv was proceeding with the contract, EPA released guidelines for safety testing and registering insect viruses. After Bio-Serve came Reuter Labs, Haymarket, VA, which promised a gypsy moth NPV product and was endorsed by Washington Office-Forest Pest Management. Their product was inferior and never was put on the market.

In late 1976, a joint FS, Agricultural Research Service (ARS), and Animal and Plant Health Inspection Service (APHIS) research and development program on mass NPV propagation was initiated. The major objective of the FS-ARS-APHIS effort was to design and test a mass NPV production method that would yield an NPV product and meet all the EPA requirements for microbial insecticides.

In 1977, an aerial field test was conducted with the new NPV product, Gypchek, produced by FS-ARS-APHIS. Results did show that egg mass densities were reduced on the average of 77 and 64 percent in the treatment plots as compared to the controls with an increase of 37 percent.

In April, 1978, Gypchek was officially approved and registered by the EPA as a general use insecticide for aerial and ground application to control gypsy moth. Registration was a long process, aided principally by the prior registration of the *Heliothis* NPV (Elcar®) (Sandoz, Wasco, CA) and to some extent by the registration of the Douglas-fir tussock moth NPV.

In the 1980's, laboratory and field studies were intensified in an effort to develop Gypchek for operational use in environmentally sensitive areas. Since Gypchek is inactivated rapidly by sunlight after application to foliage, large numbers of potential sunscreens were evaluated in order to identify a more effective UV protectant for the Gypchek tank mix. These studies did not conclusively demonstrate clear superiority of any UV protectant or tank mix.

Successful field tests awakened commercial interests. The FS entered into a technology transfer agreement with a Maryland company, Espro, Inc., with the purpose of commercializing the production of Gypchek. Another company, Calliope (Beziers, France) showed interest in Gypchek production and had produced a gypsy moth NPV product "Lymantrine®" in a flowable formulation ready for simulated field testing. The FS continued the production of Gypchek to support methods improvement studies and for using in high priority operational programs (areas containing threatened and endangered species that would be affected by other insecticides).

In 1991, Espro, Inc. merged with Crop Genetics Corporation and efforts toward the commercialization of Gypchek was discontinued. In 1992, Calliope, stopped producing Lymantrine due to the high cost of production. As gypsy moth continued to expand its range south and west, the demand for Gypchek increased dramatically, particularly for use in environmentally sensitive.

Unfortunately, product demand outweighed supply. The shortage related to several factors including (1) gypsy moth-baculovirus dynamics [gypsy moth are relatively resistant to their baculovirus], (2) production technology and costs, (3) FS policy, which is "the government is not in the business of doing business", Gypchek production is secondary to the overall research and methods development objectives of the FS and APHIS, and (4) the reluctance of industry to commit to product development.

The lack of a commercially available, ready to use product slowed the integration of Gypchek into gypsy moth management programs. Not only was the supply of Gypchek limited, but the standard tank mix was cumbersome. In 1992, the FS collaborated with American Cyanamid in the development and evaluation of a commercial wettable powder formulation of Gypchek, and with Entotech, Inc. in the development and evaluation of an aqueous flowable spray carrier for Gypchek, to replace the current "user unfriendly" tank mix. One ready-to-use formulation from each company was selected for field testing following numerous laboratory and spray tower evaluations of efficacy and weatherability.

The results with the American Cyanamid wettable powder and the Entotech aqueous flowable virus adjuvant were most encouraging, but more field tests needed to be conducted in areas of low-density, building populations. In 1993, the FS completed laboratory and spray tower evaluations on six additional formulations that are refinements of the 1992 Entotech formulation. All six were superior to the FS standard formulation in protecting Gypchek from ultra-violet light. One formulation (Entotech #109-5) retained better than 90 percent of its original activity compared to about 50 percent for the standard formulation.

In 1993, the American Cyanamid and Entotech formulations were field tested on replicated plots in Michigan. Results from the Entotech study in conjunction with the 1992 results, supported the replacement of the FS standard tank mix with the Entotech carrier for use on all operational Gypchek programs. The 1993 results for the American Cyanamid study were not as promising and in 1994 American Cyanamid discontinued production.

Extensive research and development has advanced Gypchek from a product that was once considered a "curiosity" by forest pest managers to one that is requested for use in environmentally sensitive areas in gypsy moth management programs. Demand for the product is great, but the supply is still limited (only 20,000-acre equivalents are available for 1996). While waiting for the commercial development of a Gypchek-type product, the FS and its cooperators continue to seek-out and test more potent and faster killing strains of gypsy moth NPV and field test commercial formulations and spray adjuvants as they become available. In addition, research is ongoing to develop genetically engineered strains of gypsy moth NPV.

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Field Evaluations of Gypchek/Carrier Formulations, 1994-1995

Kevin Thorpe, John Podgwaite, Ralph Webb, Richard Reardon, and Stephen Cook[1]

Introduction

The gypsy moth nucleopolyhedrosis virus product, Gypchek™, was registered with the U.S. Environmental Protection Agency in 1978 as a general use pesticide to control the gypsy moth. Because it affects only the gypsy moth, it is the ideal pesticide for use in situations where gypsy moth control is needed and concern about effects on nontarget organisms is paramount. Unfortunately, this product is not commercially available. The Forest Service (FS) currently produces a limited supply for use in research and selected operational programs. Research over the past 15 years has resulted in the improvement of Gypchek formulations and application methodology to the point where reasonably consistent foliage protection and reduction of gypsy moth larval populations can be obtained. Until recently, the FS recommended two applications of Gypchek in a tank mix consisting of lignosulfonate (a UV screen), molasses, sticker, and water at a dose of 2 X 10^{11} occlusion bodies (OBs) and a volume of 2 gallons per acre per application. While providing efficacious results, this tank mix is expensive, messy, and awkward to use. These problems are viewed as an additional obstacle to the effective operational use of Gypchek and a possible barrier to the commercial development of a viable Gypchek product. For this reason, there has been considerable interest in the development of a ready-to-use commercial carrier to replace the lignosulfonate-molasses tank mix. Beginning in 1992, experimental adjuvants, selected through lab bioassy and spray-tower tests, have been included in annual major Gypchek field research projects. The results have been promising, and the FS's recommendations now include the use of double applications of Gypchek in one gallon of commercial carrier at the same dosage as with the lignosulfonate-molasses tank mix.

Carrier Development

In 1992, Entotech, Inc., Davis, CA, a research division of Novo Nordisk of Denmark, developed a number of experimental flowable spray adjuvants that were evaluated in the laboratory by the FS for use as a carrier for Gypchek. A field test conducted in Pennsylvania that year indicated that the formulation compared favorably with the lignosulfonate-molasses tank mix. An improved version of the material, designated Carrier 244, was field tested in Michigan the following year and positive results were again realized. Field trials with Carrier 244 in Virginia in 1994 indicated that further refinement in Carrier 244 was needed to improve its mixing and handling properties. In response, Entotech developed a new carrier, Carrier 038, for the 1995 field season. This adjuvant performed well in an airport trial that was conducted in Bridgewater, VA, in November 1994.

1995 Field Test

In May 1995, an aerial application methods improvement project was designed and conducted jointly by ARS, FS, and APHIS, with the assistance of the State of Virginia. The primary objectives of the study were: 1) to compare the efficacy of the Carrier 038 formulation with the lignosulfonate-molassses tank mix; 2) to compare the efficacy of single versus double applications of the Carrier 038 formulation; and 3) to evaluate the efficacy of a reduced volume application of the Carrier 038 formulation. Thirty-six 10-acre plots were delineated within the Goshen Wildlife Management Area, Goshen, VA. Pre-season gypsy moth egg mass densities in these plots ranged from 107 to 4,815 per acre. The following five treatments were tested:

1. Double application of lignosulfonate-molasses tank mix with Gypchek at 2 X 10^{11} OBs in two gallons of formulation per acre per application.

2. Double application of Carrier 038 with Gypchek at 2 X 10^{11} OBs in one gallon of formulation per acre per application.

3. Single application of Carrier 038 Gypchek at 4 X 10^{11} OBS in one gallon of formulation per acre.

4. Double application of Carrier 038 with Gypchek at 2 X 10^{11} OBS in ½ gallon of formulation per acre per application.

5. Untreated control.

The aerial applications were conducted by APHIS using a Cessna Ag Truck fitted with 8006 flat fan nozzles. The single application treatment, and the first of two applications for the other treatments, occurred on May 6 and 7. The second applications were made on May 8 and 9.

In between the first and second applications, two additional 10-acre plots were treated for spray deposit analysis with each of the double application treatments. The recommended interval of 3 days between applications was shortened to 2 days because of a forecast of rain. Rain posed a potentially serious problem because, at the time of the experiment, a suitable sticker that was compatible with Carrier 038 had not been found. Fortunately, no rain occurred until late in the day on May 9, which was more than 24 hours after the last plot had been treated with Carrier 038. Gypsy moth larvae were predominantly in the first instar at the time of the applications. Oak leaf expansion was ca 25 percent, but was highly variable among plots.

The effectiveness of the treatments was evaluated in several ways. Pre-season virus levels were measured by determining the mortality of gypsy moth larvae hatched from egg masses collected within each of the plots and reared on artificial diet, and by collecting larvae from each plot during the week preceding the spray application, rearing them on artificial diet, and recording mortality. Treatment mortality was assessed by determining the mortality of larvae collected 10 days after spray application and reared on artificial diet. A frass sample, conducted 20 days after treatment, provided an estimate of larval population density. Estimates of defoliation provided yet another measure of treatment effects. At peak defoliation, which occurred in mid-June, workers estimated the amount of defoliation that had occurred to each of 20 trees in each plot. Finally, post-season egg mass counts will be taken after leaf fall. Shotguns were used to sample leaves from the 6 spray deposit plots; these plots were sprayed with the same treatments except that a fluorescing dye was added to facilitate quantification of spray deposit. Data from this aspect of the study are not yet available.

Results

Mortality of gypsy moth larvae from egg masses collected from the plots averaged 2.9 percent, suggesting that levels of naturally-occurring virus were low at the beginning of the season. As often occurs in gypsy moth populations, levels of naturally-occurring virus increased as the season progressed, averaging 30 percent among untreated larvae that were collected 10 days after the treatment plots were sprayed. Mortality among larvae collected at the same time from treated plots ranged from 73 percent with a single application of Carrier 038 + Gypchek to 90 percent with the high volume, double application of Carrier 038 + Gypchek. Preliminary results indicate that mortality was significantly lower among larvae collected from plots treated with only a single application. Larval density was estimated at 126 larvae per m^2 of ground surface in the untreated plots, 57 per m^2 in the single application plots, and less than 25 per m^2 in the plots treated with the other treatments. Defoliation in the control plots averaged 38 percent, which was significantly higher than the defoliation which occurred in the treated plots (15 to 23 percent).

Late-Season Population Collapse

Shortly after the frass samples were obtained, large numbers of gypsy moth cadavers began to appear in all of the plots. Many of these insects showed symptoms characteristic of mortality caused by the fungus, *Entomophaga maimaiga*. Over the next few weeks, larval numbers in the plots dropped noticeably, until by mid-June larval numbers were so low in all plots that sampling was discontinued. Necropsies conducted on larvae that were alive when collected but that died after being placed on artificial diet indicated that *Entomophaga maimaiga* was a significant source of gypsy moth mortality in all plots. For example, necropsies of larvae that were collected from a single application plot 10 days after treatment indicated that 98 percent of the cadavers contained virus but only 2 percent contained fungus. Necropsies of larvae from the same plot, but collected two weeks later revealed that 23 percent of the cadavers contained virus and 92 percent contained fungus. It is likely that the defoliation levels would have been higher in the control plots (and perhaps also in some of the treatment plots) if late-season mortality from *Entomophaga maimaiga* had not been so severe. Fortunately, useful results were obtained from the 10-day post-treatment larval collection data and frass count data, which were collected prior to the major collapse of the populations.

Conclusion

Although data collection and analysis are not complete, some conclusions are possible at this time. First, Carrier 038 exhibited excellent physical properties under operational conditions. It mixed and flowed well through standard application equipment, and exhibited good spray deposit characteristics. All of the treatments tested resulted in higher gypsy moth mortality and less foliage damage than occurred in the control plots. There was no detectable difference between the performance of the lignosulfonate-molasses tank mix and the double application of Carrier 038 + Gypchek at either the 1 gallon or the ½ gallon volume. While the single application of Carrier 038 + Gypchek provided good

foliage protection, there were indications that larval mortality may have been somewhat lower under this treatment. Further field trials will be necessary to confirm these results. Our results support the current FS recommendation that a double application of Carrier 038 + Gypchek at one gallon of formulation and 2 x 10^{11} OBs per acre per application will provide gypsy moth control that is comparable to that obtained with a double application of the standard lignosulfonate-molasses tank mix at 2 gallons per acre per application. Furthermore, there was no evidence in this study of reduced efficacy with a double application of Carrier 038 + Gypchek at the ½ gallon rate. If this can be confirmed in future methods improvement tests, the operational use of a lower volume could lead to a further savings in cost of materials and application.

[1]*Kevin is employed with the Agricultural Research Service in Beltsville, MD; John is an employee with the USDA Forest Service in Hamden, CT; Ralph is employed with APHIS in Beltsville, MD; Dick is employed with the USDA Forest Service in Morgantown, WV; and Stephen is employed with the University of Oklahoma in Norman, OK.*

Letter to the Editor

B. H., Richmond, VA, writes:

"Where can I find information concerning the pesticides currently in use for the control of Gypsy Moth? I am particularly interested in the control areas around homes. Any help in this matter would be greatly appreciated."

Noel Schneeberger from the USDA Forest Service, Northeastern Area State and Private Forestry in Radnor, PA, responds:

Three insecticides are commonly used in gypsy moth suppression and eradication projects conducted by State and local governments in cooperation with the USDA Forest Service, and USDA Animal and Plant Health Inspection Service (APHIS): Bacillus thuringiensis variety kurstaki (B.t.k.), diflubenzuron, and Gypchek. Cooperative projects generally involve aerial application of one of these insecticides over forested residential areas, recreation areas, and forests.

A detailed examination of these three insecticides as well as other treatments is presented in an environmental impact statement (EIS)--Gypsy Moth Management in the United States: a cooperative approach. The EIS presents information about how these insecticides are used, how effective they are, the environmental consequences of using them, and the human health risks associated with exposure to them.

Other insecticides are also registered and available to the homeowner. These include formulations containing carbaryl (Sevin®), and acephate (Orthene). A local garden supply or hardware store should carry these products. A recent publication may also answer your questions. "Homeowner's Guide to Gypsy Moth Management" by Emily Grafton and Dr. Ralph Webb was published by the West Virginia University Extension Service. The guide presents information on insecticidal and noninsecticidal means to control gypsy moth. For a copy of either the new EIS, the homeowner "Guide", or both, contact me at 610-975-4121.